CRYSTAL STRUCTURE OF N^6-(Δ^2-ISOPENTENYL)ADENINE, A BASE IN THE ANTICODON LOOP OF SOME tRNA's

Charles E. Bugg
Institute of Dental Research and Department of Biochemistry, University of Alabama in Birmingham, 1919 Seventh Avenue, South Birmingham, Alabama 35233

Ulf Thewalt
Mineralogisches Institut der Universität, 355 Marburg/Lahn, Deutschhausstr. 10, West Germany

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SUMMARY: The crystal structure of N^6-(Δ^2-isopentenyl)adenine was determined from three-dimensional X-ray diffraction data. The isopentenyl moiety is pointing away from the adenine imidazole ring, and assumes a conformation that prevents the N(1) position of adenine from accepting hydrogen bonds; consequently, the two sites, N(6)-H and N(1), that are normally utilized by adenine bases for complementary pairing within double helical regions of nucleic acids are blocked by the isopentenyl sidechain. The observed structure is consistent with the hypothesis that this purine derivative plays a role in maintaining the anticodon loop in a single stranded conformation that enhances codon-anticodon interactions.

The purine derivative N^6-(Δ^2-isopentenyl)adenine (IPA, Figure 1) is a minor constituent of some tRNA's (1,2). This

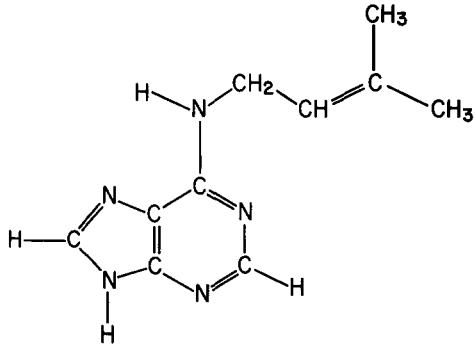

FIGURE 1. Structural Formula of N^6-(Δ^2-isopentenyl)adenine.

unusual base seems to occur only in those tRNA's that contain an adenosine residue in the 3' terminus of the anticodon triplet, and it is always found immediately adjacent to the 3' end of the anticodon (2). Little is known about the possible function of IPA in tRNA, but there is evidence that its presence results in enhanced codon-anticodon interactions (2,3,4). It is possible that the isopentenyl moiety assumes a conformation in which the bulky, hydrophobic sidechain affects base stacking and hydrogen bonding within tRNA, thus enhancing interactions with mRNA. We describe here the conformation, hydrogen bonding scheme and base stacking pattern found in the crystal structure of IPA.

METHODS

Crystals of IPA were grown by evaporating a methanol-butyl ether solution at room temperature. The compound crystallizes in space group $P\bar{1}$ with unit cell dimensions a = 4.9834(4), b = 13.427(1), c = 7.869(1) Å and α = 97.57(1), β = 100.52(1), γ = 92.70(1)°. Intensities for the 1684 unique reflections with $2\theta \leq 128^{\circ}$ were measured on an automated diffractometer using nickel-filtered copper radiation. Trial coordinates for the nine atoms of the purine moiety plus the amino nitrogen atom were obtained from a sharpened, three-dimensional Patterson map; the remaining nonhydrogen atoms were located in a Fourier map calculated by using phase angles derived from these ten atoms. Coordinates of the nonhydrogen atoms, along with anisotropic temperature factors, a scale factor and an extinction correction parameter, were refined by full-matrix least-squares. All hydrogen atoms were located in a difference Fourier map calculated during the latter stages of refinement. The hydrogen atom positional parameters and isotropic temperature factors were included in the last cycles of least-squares heavy atom and hydrogen atom parameters were refined in alternate

cycles. An electron density difference map calculated after convergence showed no peaks or troughs exceeding 0.2 e/Å^3 in magnitude.

The final R index ($=\Sigma||Fo|-|Fc||/\Sigma Fo$) for all reflections is 0.048. Table 1 lists atomic positional parameters and their estimated standard deviations. The average estimated deviations in the positional coordinates of the heavy atoms are 0.001-0.003 Å, and those for the hydrogen atoms are 0.01-0.03 Å. Additional experimental details, a table of atomic thermal parameters, and a table of observed and calculated structure factors will be furnished upon request.

TABLE 1. Final atomic positional parameters and their standard deviations.

ATOM	x	y	z	ATOM	x	y	z
N(1)	.5614(3)	.2331(1)	.3263(2)	H(2)	.237(3)	.271(1)	.177(2)
C(2)	.3284(4)	.2133(1)	.2079(2)	H(6)	1.022(3)	.117(1)	.529(2)
N(3)	.2043(3)	.1256(1)	.1281(2)	H(8)	.496(3)	-.173(1)	.201(2)
C(4)	.3474(3)	.0487(1)	.1818(2)	H(9)	.139(3)	-.083(1)	.050(2)
C(5)	.5902(3)	.0564(1)	.3015(2)	H(161)	1.242(3)	.271(1)	.595(2)
C(6)	.6994(3)	.1541(1)	.3772(2)	H(261)	.983(3)	.321(1)	.497(2)
N(7)	.6795(3)	-.0379(1)	.3223(2)	H(62)	1.021(3)	.258(1)	.840(2)
C(8)	.4903(3)	-.0990(1)	.2161(2)	H(164)	.527(4)	.385(1)	.955(3)
N(9)	.2854(3)	-.0520(1)	.1284(2)	H(264)	.800(4)	.336(1)	1.046(3)
N(6)	.9339(3)	.1710(1)	.4947(2)	H(364)	.784(4)	.456(2)	1.035(3)
C(61)	1.0453(3)	.2714(1)	.5791(2)	H(165)	.718(5)	.425(2)	.559(4)
C(62)	.9565(3)	.2990(1)	.7498(2)	H(265)	.533(6)	.466(2)	.692(4)
C(63)	.8053(3)	.3735(1)	.7939(2)	H(365)	.823(7)	.509(2)	.702(4)
C(64)	.7220(4)	.3872(2)	.9684(3)				
C(65)	.6999(5)	.4475(2)	.6755(3)				

RESULTS AND DISCUSSION

Figure 2 shows the conformation of IPA, along with the bond lengths and angles involving nonhydrogen atoms. IPA crystallizes as the N(9)-H tautomer. The purine moiety is essentially planar with no atomic deviations in excess of 0.01 Å. The amino nitrogen atom, N(6), and its immediate substituents are also practically

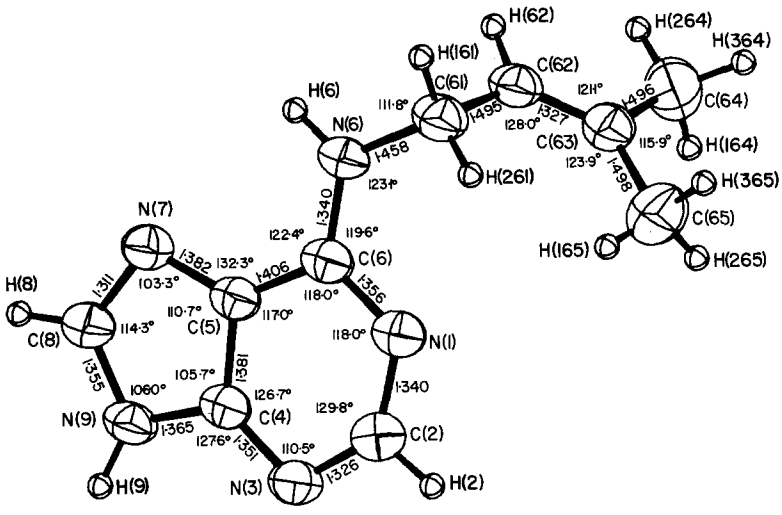

FIGURE 2. Conformation of N^6-(Δ^2-isopentenyl)adenine. The nonhydrogen atoms are represented by thermal ellipsoids scaled to include 50% probability. Only those bond lengths and angles involving nonhydrogen atoms are shown; the estimated errors are about 0.004 Å in bond lengths and 0.2° in bond angles. This drawing was prepared using the computer program ORTEP (5).

coplanar with the purine ring. The conformation around the C(6)-N(6) bond is such that the isopentenyl moiety points away from the imidazole ring. In the crystal structures of N^6-{2-(4-imidazolyl)-ethyl}adenine (6) and N^6-(Δ^2-isopentenyl)-2-methylthioadenine (2-MT-IPA) (7) the sidechains bonded to the amino group also point away from the imidazole rings of the adenine moieties. As expected, atoms C(61), C(62), H(62), C(63), C(64) and C(65) are nearly coplanar; the plane defined by these atoms forms a dihedral angle of 72° with the plane of the purine ring. This conformation is different from that of 2-MT-IPA in which the isopentenyl moiety is inclined 91° with respect to the purine. The major difference between IPA and 2-MT-IPA is in the conformation about the C(61)-C(62) bond: in conformational projections the C(62)-H(62) bond of IPA bisects the H(161)-C(61)-N(6) angle, while in 2-MT-IPA this bond bisects the H(261)-C(61)-N(6) angle. The conformation of 2-MT-IPA probably differs from that of IPA because of steric repulsion between the 2-methylthio substituent

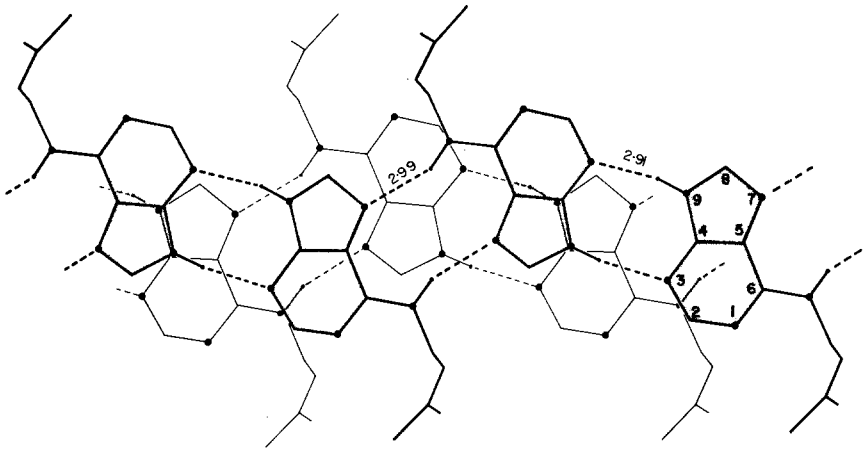

FIGURE 3. The hydrogen bonding scheme and base stacking pattern. Only those hydrogen atoms involved in hydrogen bonding are shown. The planes of purine bases are about 3.3 Å apart.

and the isopentenyl moiety.

The crystal packing is depicted in Figure 3. The bases are linked across crystallographic inversion centers by N(9)-H---N(3) and N(6)-H---N(7) hydrogen bonds resulting in continous ribbons of purine bases. This hydrogen bonding pattern is essentially the same as that in the crystal structure of 2-MT-IPA. As found (8) in numerous crystal structures of adenine derivatives that lack N(6) substituents, the base stacking pattern involves only slight overlap of bases and atom N(6) is positioned above the ring system of an adjacent purine. The isopentenyl moieties are extended away from the purine rings to form hydrophobic regions surrounding the stacked ribbons.

It is noteworthy that atom N(1) fails to accept a hydrogen bond in this crystal structure. Due to the conformation of the bulky isopentenyl moiety, N(1) is completely shielded and is inaccessible to possible hydrogen bond donors. In addition, since the sidechain is directed away from the imidazole ring, the amino group can only form hydrogen bonds on the backside of the purine ring. Consequently, the two sites, N(1) and N(6)-H, that are normally utilized by adenine bases for complementary

pairing within double helical nucleic acids are blocked by the isopentenyl substituent. This implies that the isopentenyl adenine moieties of tRNA would be unlikely to occur within base paired, double-helical segments. Therefore, IPA seems to be ideally suited for occupying the position adjacent to the 3' end of the anticodon triplet, a position that is not involved in base pairing with other tRNA residues when the anticodon loop is in the conformation postulated by Fuller and Hodgson (9). It is likely that IPA plays a role in maintaining the anticodon loop in a single stranded conformation that enhances codon-anticodon interactions.

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1. Abbreviations Used: IPA, N^6-(Δ^2-isopentenyl)adenine; 2-MT-IPA, N^6-(Δ^2-isopentenyl)-2-methylthioadenine; tRNA, transfer ribonucleic acid; mRNA, messenger ribonucleic acid.
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